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Serum-free insulin-like growth factor I correlates with clearance in patients with chronic renal failure

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Background. Chronic renal failure (CRF) results in major changes in the circulating growth hormone (GH)/insulin-like growth factor (IGF) system. However, there are only limited data on changes in free IGF-I in CRF.

Methods. Matched groups of nondiabetic, nondialyzed patients with CRF ($N = 25$) and healthy controls ($N = 13$) were compared. The creatinine clearance (C_{Cr}) based on a 24-hour urine collection ranged from 3 to 59 and 89 to 148 ml/min/1.73 m^2 in patients and controls, respectively. Overnight fasting serum samples were analyzed for free and total IGF-I and -II, and IGF-binding protein (IGFBP)-1, -2, and -3. Additionally, intact as well as proteolyzed IGFBP-3 was determined.

Results. The patients had reduced serum-free IGF-I (−53%) and increased levels of total IGF-II (40%), IGFBP-1 (546%), and IGFBP-2 (270%, $P < 0.05$). Serum total IGF-I and free IGF-II were normal. Also, serum levels of immunoreactive IGFBP-3 were elevated (33%, $P < 0.05$), but this could be explained by an increased abundance of IGFBP-3 fragments, as ligand blotting showed no difference in levels of intact IGFBP-3. Accordingly, patients had an increased proteolysis of IGFBP-3 *in vivo* (17%) and *in vitro* (7%, $P < 0.05$). In patients, free IGF-I levels correlated positively with C_{Cr} ($r^2 = 0.38$, $P < 0.002$) and inversely with IGFBP-1 ($r^2 = 0.69$, $P < 0.0001$) and IGFBP-2 ($r^2 = 0.41$, $P < 0.0007$), whereas C_{Cr} was inversely correlated with levels of IGFBP-1 ($r^2 = 0.48$, $P < 0.0001$) and IGFBP-2 ($r^2 = 0.63$, $P < 0.0001$).

Conclusions. These data strongly support the hypothesis that CRF-related growth failure and tissue catabolism are caused by an increased concentration of circulating IGFBP-1 and -2, resulting in low serum levels of free IGF-I and thus IGF-I bioactivity. In addition, low levels of free IGF-I may explain the increased secretion of GH in CRF.

Key words: growth hormone, tissue catabolism, end-stage renal failure, creatinine clearance.

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Growth retardation and muscle protein catabolism are common features in patients suffering from chronic renal failure (CRF), and numerous clinical as well as experimental studies suggest abnormalities in the growth hormone (GH)/insulin-like growth factor (IGF) system to play a key role [1, 2]. Patients with CRF have elevated serum levels of GH [3] that appear to be a consequence of an increased secretion rate and reduced metabolic clearance rate (MCR) [2, 4, 5]. Concomitantly, CRF is characterized by reduced circulating levels of the high-affinity GH-binding protein (GHBP), which has been used as an indicator of the hepatic GH receptor density [6]. The GH hypersecretion in CRF is therefore believed to reflect a state of hepatic GH resistance [2].

In addition to GH resistance, the bioactivity measured *in vitro* of the IGF system appears to be reduced in CRF [7–9]. Because levels of serum total (extractable) IGF-I are usually within the normal range [1], interest has focused on changes in the circulating levels of the IGF-binding proteins (IGFBPs) [7, 8]. Thus, patients with CRF have elevated serum levels of IGFBP-1, IGFBP-2, and IGFBP-4 [10, 11], which are all predominantly inhibitory to the actions of IGF-I [12, 13]. Additionally, CRF is associated with elevated serum levels of low molecular fragments of IGFBP-3, some of which have retained the ability to bind IGF-I, albeit with lower affinity [14]. The accumulation of circulating IGFBPs and IGFBP fragments is believed to play a central role in the growth abnormalities observed in patients with CRF, and this view has been supported by *in vitro* as well as *in vivo* data. Blum et al showed that the removal of excess IGFBPs in serum from patients with CRF normalized the *in vitro* IGF-bioactivity [7], and Tönshoff et al found an inverse correlation between height and serum levels of IGFBP-1 as well as IGFBP-2 in a cross-sectional study of prepubertal children with CRF [10]. Taken together, these observations strongly suggest that the circulating levels of free and presumably bioactive IGF-I are reduced in

Table 1. Subject characteristics

Variables	Controls	Patients	*P*
N	13	25	
Males/females	8/5	18/7	NS
Age *years*	46 ± 2	51 ± 2	NS
BMI kg/m^2	24.7 ± 1.2	24.5 ± 1.1	NS
Creatinine clearance $ml/min/1.73\ m^2$ (range)	113 ± 4 (89–148)	26 ± 3 (3–59)	< 0.0001
Fasting serum glucose *mmol/liter*	4.6 ± 0.3	5.1 ± 0.2	NS
Serum albumin *g/liter*	44 ± 1	35 ± 1	< 0.0001

Data are mean ± SEM, and range when appropriate.

CRF [7]. Low serum levels of free IGF-I may at the same time contribute to the increased secretion of GH and some of the growth abnormalities seen in patients with CRF.

The aim of this study was to test the hypothesis that levels of free IGF-I are reduced in patients with CRF and that this reduction is related to an accumulation of circulating IGFBPs. We also wanted to investigate whether the CRF-related changes in the IGF system are associated with the severity of disease. Therefore, we selected the patients so that their kidney function, expressed as clearance of creatinine (C_{Cr}), ranged from incipient CRF to overt end-stage renal failure (ESRF).

METHODS

Subjects

The study included 25 nondiabetic patients with CRF and 13 healthy controls matched for age, gender, and body mass index (BMI; Table 1). All patients were recruited from the Department of Nephrology, Aarhus University Hospital, Denmark. They were newly diagnosed, and none of the patients had received any treatment with dialysis prior to the time of blood sampling. The presence of renal disease was based on routine physical examination, biochemical findings and renal biopsy, histology, or, in the case of adult polycystic kidney disease, ultrasound. The patients were diagnosed as having glomerulonephritis ($N = 7$), nephrosclerosis and hypertensive nephropathy ($N = 6$), adult polycystic kidney disease ($N = 6$), interstitial nephropathy ($N = 3$), IgA nephritis ($N = 1$), and unknown nephropathy ($N = 2$). Patients with CRF received oral treatment with loop diuretics ($N = 10$), thiazides ($N = 7$), calcium channel antagonists ($N = 9$), angiotensin-converting enzyme inhibitors ($N = 10$), β-blockers ($N = 10$), vitamin D ($N = 2$), calcium carbonate ($N = 5$), and erythropoietin ($N = 2$). None of the patients had received recombinant human (rh) GH, glucocorticoids, or immunosuppressive drugs. The C_{Cr} was based on a 24-hour urine collection and was expressed as ml/min/1.73 m^2. In order to study the relationship between kidney function and changes in the IGF system, patients were selected with a kidney function, expressed as C_{Cr}, ranging from incipient CRF to overt ESRF, whereas all controls had a clearance above 80 ml/min/1.73 m^2 (Table 1). The healthy controls were recruited among the hospital staff. They all underwent routine physical examination and a biochemical screening to preclude the presence of malignancy and infectious diseases. With the exception of oral contraceptives, none of the healthy controls received any prescribed medication. The study was approved by the local ethical committee, and all subjects gave informed consent to participate.

Analytical determinations

Blood samples were collected after an overnight fast, with medication being withdrawn from the night before sampling. Serum was stored at −20°C. All measurements were performed in duplicates within the same run unless otherwise stated. IGF-I and -II were determined by in-house noncompetitive monoclonal antibody-based time-resolved immunofluorometric assays (TR-IFMAs), as previously described [15]. These assays are characterized by high sensitivity [the detection limit was 2.5 ng/liter (IGF-I) and 10 ng/liter (IGF-II)] and specificity (the IGF-I and IGF-II cross reactivity in heterologous assays was < 0.0002%).

Serum total (extractable) IGF-I and -II were determined in acid ethanol serum extracts with a within assay coefficient of variation (CV) averaging less than 5% [15]. However, acid ethanol extraction may not always be sufficient to remove the IGFBPs present in serum from patients with severely affected kidney function (that is, uremia) [16], and we therefore modified the extraction procedure using the IGF-blocking principle originally described by Blum, Ranke, and Bierich [17]. Thus, acid ethanol serum extracts used for determination of IGF-I were neutralized in buffer containing an approximate 40-fold molar excess of IGF-II, whereas extracts used for IGF-II determinations were neutralized in buffer containing an approximate 10-fold molar excess of IGF-I. The same buffers were used for the respective calibration curves, and they caused no cross-reaction.

Serum-free IGF-I and -II were determined using ultrafiltration by centrifugation at conditions approaching those *in vivo* [18]. Amicon YMT 30 membranes and MPS-1 supporting devices were used (Amicon Division, Beverly, MA, USA). Before centrifugation, serum samples were diluted (1 in 11) in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 50 g/liter human serum albumin (Behring AG, Marburg, Germany). From each dilution, triplicates of 600 μl were applied to the membranes and incubated (30 min at 37°C) and centrifuged (1500 r.p.m. at 37°C; model Rotixa/RP; Hettich Zentrifugen, Tuttlingen, Germany). The lower detection limit of free IGF-I and -II in the ultrafiltrates was 20 and 90 ng/liter, respectively. Levels of free IGF-I and -II were analyzed

in separate ultrafiltrates. Including ultrafiltration and immunoassay, the within assays CV averaged 18% and 12% for free IGF-I and -II, respectively.

Insulin-like growth factor-binding proteins were determined by specific immunoassays, ligand blotting, and immunoblotting. Immunoreactive levels of IGFBP-1, -2, and -3 were determined by enzyme-linked immunosorbent assay (Medix Biochemica, Kainiainen, Finland), radioimmunoassay, and immunoradiometric assay (IRMA), respectively (both Diagnostic System Laboratories Inc., Webster, TX, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and ligand blotting were performed as originally described by Hossenlopp et al [19]. Two microliters of serum were subjected to SDS-PAGE (10% polyacrylamide) under nonreducing conditions. rhIGF-I (Amersham, Buckinghamshire, UK) was iodinated using the chloramine-T method and served as ligand (approximately 100,000 CPM per sample). The resulting autoradiograms were scanned using a laser densitometer (Shimadzu model CS 90001PC; Shimadzu Europe GmbH, Duisburg, Germany), and the relative densities of the bands were expressed in arbitrary absorbency units per square millimeter (pixel density). Serum samples from controls and patients were equally distributed on the gels.

In addition to determination of IGFBP-3 by immunoassay (immunoreactive IGFBP-3) and ligand blotting (intact 38 to 42 kDa IGFBP-3), we also estimated the *in vivo* proteolysis of IGFBP-3 by immunoblotting performed as previously described using a polyclonal IGFBP-3 antibody (Upstate Biotechnology Inc., Lake Placid, NY, USA) [20]. The immunoblot yielded three distinct bands representing intact (38 to 42 kDa) and fragmented (16 and 30 kDa) IGFBP-3 [20]. Based on the pixel density of these bands, the fraction of proteolyzed IGFBP-3 was calculated as the ratio of fragmented IGFBP-3 (30 plus 16 kDa) divided by the sum of all three IGFBP-3 bands.

In vitro proteolysis was performed as originally described by Lamson, Giudice, and Rosenfeld [21] using iodinated rhIGFBP-3 (^{125}I-rhIGFBP-3; Diagnostic System Laboratories Inc.). Two microliters of serum were incubated with approximately 30,000 CPM of ^{125}I-rhIGFBP-3 for 18 hours at 37°C and were subjected to SDS-PAGE and autoradiography. Serum samples from a healthy nonpregnant subject and a term-pregnant woman were included as internal controls. The fraction of degraded ^{125}I-rhIGFBP-3 was calculated in the same way as described earlier in this article, that is, as the ratio of the density of fragmented ^{125}I-rhIGFBP-3 divided by the sum of all ^{125}I-rhIGFBP-3–related densities. The in-between assay CV of the two control samples averaged 10%.

Insulin and GH were determined by commercial TR-IFMAs (Wallac Oy, Turku, Finland), and serum glucose was determined by the glucose-oxidase method.

Statistics

Fisher's exact test was used to compare the ratio of males versus females in the two groups. Patients and controls were compared by use of Student's unpaired *t*-test (parametric data) or the Mann–Whitney's rank sum test (nonparametric data). Linear regression analysis was performed on log-transformed data to fulfill the criteria for normality and variance homogeneity of the residuals. All data are given as mean ± SEM. A P value <0.05 was considered statistically significant.

RESULTS

The patients with CRF were characterized by increased fasting insulin (55%, $P < 0.05$; Fig. 1A) and GH (360%, $P < 0.005$; Fig. 1B) when compared with controls. Still, the patients had normal fasting serum glucose (Table 1). Serum albumin was reduced by 20% ($P < 0.0001$) in CRF when compared with controls (Table 1). No significant difference in levels of serum total IGF-I was observed (Fig. 1C), whereas serum total IGF-II was increased by 40% in the patients ($P < 0.005$; Fig. 1D). Serum-free IGF-I was markedly reduced in the patients when compared with controls (190 ± 30 vs. 400 ± 50 ng/liter, $P < 0.0005$; Fig. 1E), whereas no difference in serum-free IGF-II was observed (patients vs. controls: 1220 ± 110 vs. 1110 ± 50 ng/liter; Fig. 1F).

The patients were characterized by highly elevated serum levels of immunoreactive IGFBP-1 (546%, $P < 0.0001$; Fig. 2A) and IGFBP-2 (270%, $P < 0.0001$; Fig. 2B). Also, serum levels of immunoreactive IGFBP-3 were increased in the patients (33%, $P < 0.001$; Fig. 2C). However, this may be explained by an increased concentration of small (16 and 30 kDa) immunoreactive fragments of IGFBP-3 rather than elevated levels of intact IGFBP-3. Thus, the densities of intact 38 to 42 kDa IGFBP-3 determined by ligand blotting did not differ between the two groups (Fig. 2D), whereas IGFBP-3 immunoblotting showed an increased density of *in vivo* degraded IGFBP-3 in the patients (17%, $P < 0.001$; Fig. 2E). Also, the *in vitro* degradation of IGFBP-3 was increased in the patients (7%, $P < 0.05$; Fig. 2F).

In addition to the band at 38 to 42 kDa, which was identified as IGFBP-3 by immunoblotting, ligand blotting yielded three other distinct bands with estimated molecular weights of 33, 29, and 24 kDa. These IGFBPs were not identified by immunoblotting, but in all cases, the densities of the bands were markedly increased in patients with CRF. Thus, the pixel densities were (patients vs. controls) 229 ± 27 versus 59 ± 9 (33 kDa, $P < 0.0001$), 82 ± 11 versus 38 ± 4 (29 kDa, $P < 0.005$), and 43 ± 6 versus 24 ± 2 (24 kDa, $P < 0.005$).

We have previously identified the 29 kDa band as IGFBP-2 by immunoblotting [22], and a highly significant positive correlation was observed between serum

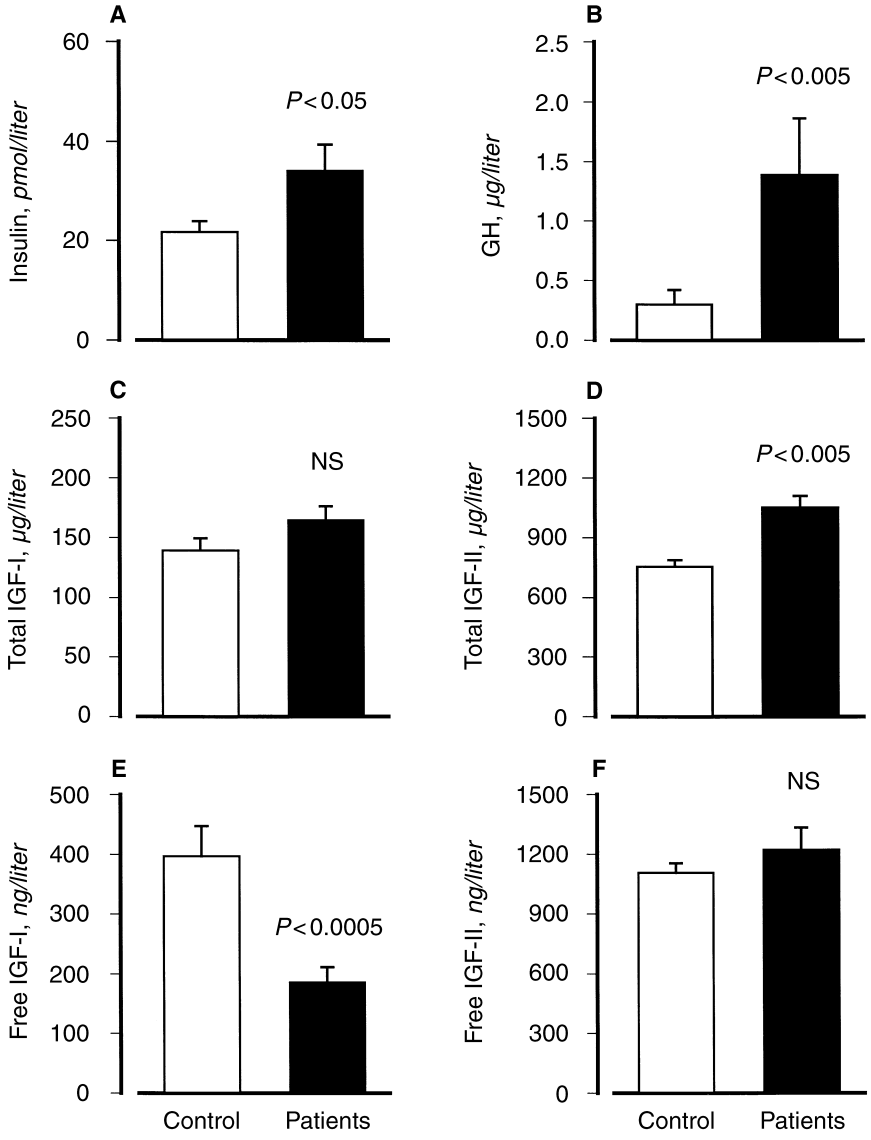

Fig. 1. Fasting serum levels of insulin (*A*), growth hormone (GH; *B*), total insulin-like growth factor-I (IGF-I; *C*), total IGF-II (*D*), free IGF-I (*E*), and free IGF-II (*F*) in controls (□) and patients (■). *P* values are indicated. NS, not significant. Data are mean ± SEM.

levels of immunoreactive IGFBP-2 and the density of the 29 kDa band ($r^2 = 0.72$, $P < 0.0001$, $N = 38$). Similarly, levels of immureactive IGFBP-1 correlated positively with the density of the 29 kDa band ($r^2 = 0.60$, $P < 0.0001$, $N = 38$), which most likely represents IGFBP-1 [1].

Linear regression analysis was used to assess the relationship between kidney function (that is, C_{Cr}) and the different members of the IGF system (Table 2 and Fig. 3). In patients with CRF, C_{Cr} correlated inversely with serum levels of IGFBP-1 and IGFBP-2 and positively with serum-free IGF-I (Table 2 and Fig. 3). An inverse correlation between C_{Cr} and the proteolysis of IGFBP-3 *in vivo* and *in vitro* was also observed (Table 2). Similar observations were made when the two groups were pooled (Table 2 and Fig. 3), whereas in controls, C_{Cr} correlated significantly only with serum IGFBP-2. Significant correlations were observed between levels of free IGF-I and total IGF-I, IGFBP-1 and IGFBP-2, respectively, whereas free IGF-I was independent of IGFBP-3 proteolysis *in vivo* as well as *in vitro*. Multiple linear regression analysis on pooled data showed that the combination of total IGF-I, IGFBP-1, and IGFBP-2 could explain more than 75% of serum levels of free IGF-I ($r^2 = 0.78$, $P < 0.0001$). Finally, in patients, levels of IGFBP-1 and insulin were inversely correlated (Table 2).

DISCUSSION

To study the impact of CRF on the circulating levels of free IGF-I and its regulatory proteins, we compared adult patients with CRF who had received no treatment with dialysis, and sex-, age- and BMI-matched controls. The study showed that adult patients with CRF had reduced overnight fasting serum levels of free IGF-I and

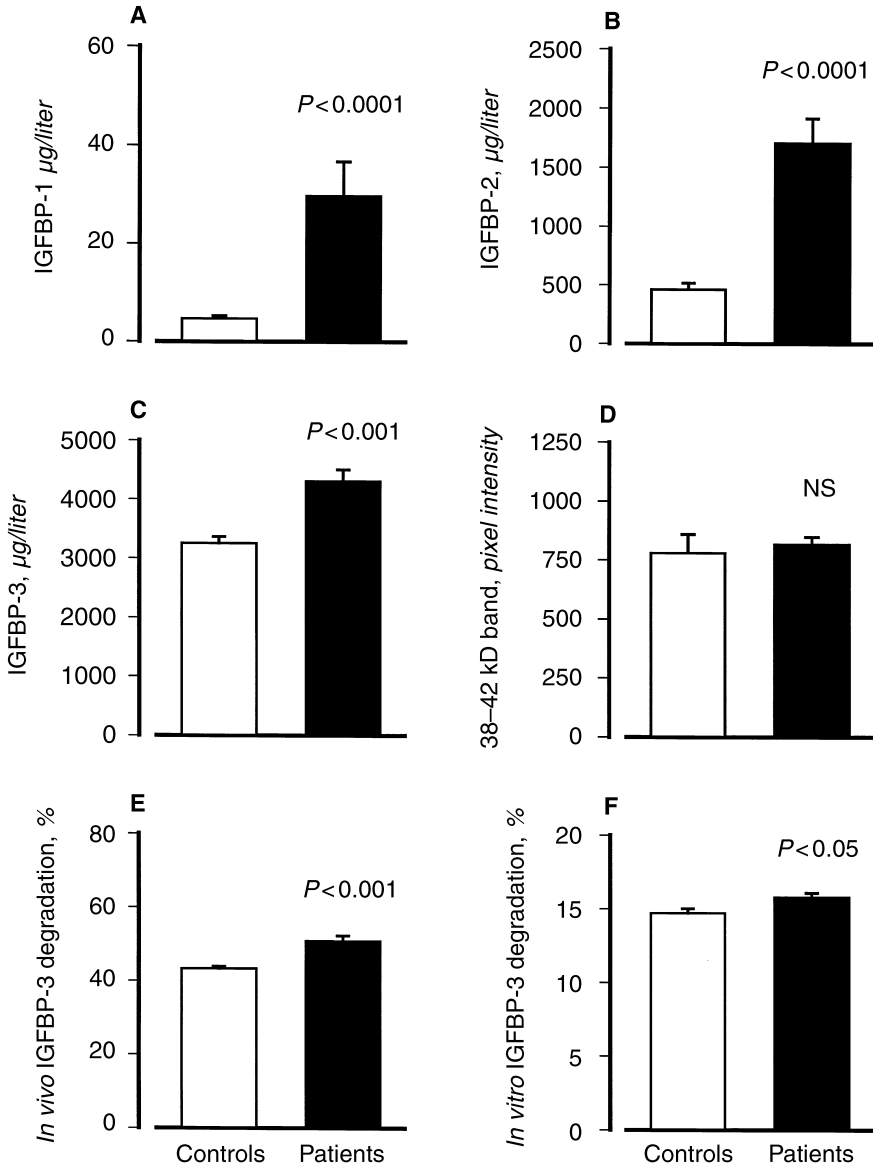

Fig. 2. Fasting serum levels of insulin-like growth factor binding protein (IGFBP)-1 (*A*), IGFBP-2 (*B*), and IGFBP-3 (*C*) determined by specific immunoassays in controls (□) and patients (■). The concentration of intact 38 to 42 kDa IGFBP-3 was also determined by ligand blotting (*D*). IGFBP-3 immunoblotting was used to estimate the fraction of *in vivo* degraded IGFBP-3 in serum (*E*). An IGFBP-3 protease assay was used to estimate the *in vitro* degradation of ^{125}I-rhIGFBP-3 in serum (*F*). *P* values are indicated. NS, not significant. Data are mean ± SEM.

Table 2. Linear regression analysis

Independent vs. dependent variable	Controls (N = 13)	Patients (N = 25)	Both groups (N = 38)
Clearance vs. IGFBP-1	NS	$P<0.0001$, $r^2=0.48$	$P<0.0001$, $r^2=0.69$
Clearance vs. IGFBP-2	$P<0.02$, $r^2=0.46$	$P<0.0001$, $r^2=0.63$	$P<0.0001$, $r^2=0.80$
Clearance vs. free IGF-I	NS	$P<0.002$, $r^2=0.38$	$P<0.0001$, $r^2=0.50$
Clearance vs. IGFBP-3 *in vivo* degradation[a]	NS	$P<0.0005$, $r^2=0.45$	$P<0.0001$, $r^2=0.60$
Clearance vs. IGFBP-3 *in vitro* degradation[a]	NS	$P<0.05$, $r^2=0.17$	$P<0.002$, $r^2=0.25$
Insulin vs. IGFBP-1	NS	$P<0.0005$, $r^2=0.41$	NS
IGFBP-1 vs. free IGF-I	NS	$P<0.0001$, $r^2=0.69$	$P<0.0001$, $r^2=0.68$
IGFBP-2 vs. free IGF-I	$P<0.03$, $r^2=0.34$	$P<0.0007$, $r^2=0.41$	$P<0.0001$, $r^2=0.50$
Total IGF-I vs. free IGF-I	$P<0.005$, $r^2=0.54$	$P<0.01$, $r^2=0.29$	$P<0.02$, $r^2=0.15$
IGFBP-3 *in vivo* degradation vs. free IGF-I	NS	NS	$P<0.007$, $r^2=0.20$
IGFBP-3 *in vitro* degradation vs. free IGF-I	NS	NS	$P<0.02$, $r^2=0.15$

All data were log transformed prior to analysis to improve normality and variance homogeneity.
Abbreviations are: IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; NS, not significant.
[a] Due to the limited capacity of the gels, the number of samples was reduced to 12 (controls) and 24 (patients); the two omitted samples were randomly selected

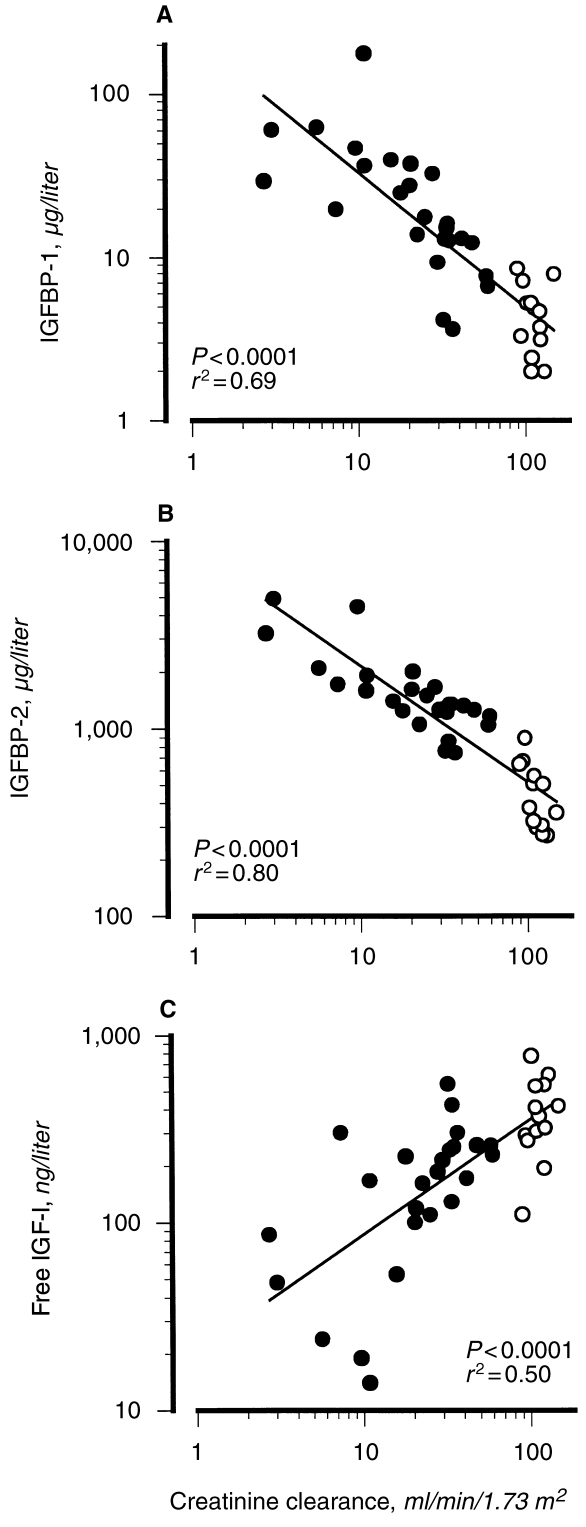

Fig. 3. Linear regressions between creatinine clearance and serum levels of IGFBP-1 (*A*), IGFBP-2 (*B*), and free IGF-I (*C*). Please note the log scales of the *X* and *Y* axes. The regression lines are based on pooled data of controls (○) and patients (●). *P* and *r* values are indicated.

increased levels of intact IGFBP-1 and -2. Also, serum levels of IGFBP-3 were increased, but this could be explained by an elevated concentration of IGFBP-3 fragments, as the density of intact IGFBP-3 was unchanged. Accordingly, patients with CRF showed a significantly increased *in vivo* and *in vitro* proteolysis of IGFBP-3. However, this appeared to be without any significance for levels of free IGF-I. Instead, more than 75% of the variance in serum-free IGF-I in CRF could be predicted from a linear combination of levels of IGFBP-1, IGFBP-2, and total IGF-I. It is noteworthy that the kidney function, expressed as C_{Cr}, correlated positively with free IGF-I and inversely with IGFBP-1 and -2. Finally, levels of free IGF-II remained within the normal range in CRF, whereas serum total IGF-II was significantly elevated.

In our study, serum-free IGF-I was determined by ultrafiltration at conditions approaching those *in vivo*, that is, at 37°C and pH 7.4 [18], and employing this technique, we observed a reduction of more than 50% in serum levels of free IGF-I in patients with CRF. This observation is different from two previous studies. Rabkin et al compared serum levels of free (plus readily dissociable) IGF-I in groups of adults ($N = 6$) with CRF and matched healthy controls before and after a subcutaneous injection of rhIGF-I (80 µg/kg) [23]. However, they failed to observe any significant difference in either baseline levels or in the increase in free IGF-I following injection, despite elevated serum IGFBP levels in CRF. Bereket et al compared serum levels of free (plus readily dissociable) IGF-I in children with ESRF ($N = 5$) before and after one year of GH treatment [24]. Although levels increased approximately 2.5-fold following GH therapy, the pretreatment level of free IGF-I was not significantly different from that of matched controls. Most likely, the discrepancy between our findings and those of Rabkin et al and Bereket et al is explained by the use of different assays for free IGF-I [23, 24]. Thus, both said investigations employed a commercially available IRMA based on an immobilized antibody directed against free IGF-I (obtained from Diagnostic System Laboratories Inc.) [23, 24]. This assay is performed by incubating serum samples directly in antibody-coated test tubes for two hours at 5°C. After incubation and wash, a second ^{125}I-labeled IGF-I antibody was used for detection. The IRMA is believed to overestimate the concentration of free IGF-I, as the solid-phase antibody most likely extracts some "loosely bound" IGF-I from the low affinity IGFBPs or IGFBP fragments [23]. In the case of CRF, the concentration of low molecular IGFBP-3 fragments is significantly increased [2], and although some of them are still able to bind IGF-I, the affinity is much lower than that of intact IGFBP-3 [14, 25, 26]. Therefore, in abnormal situations such as CRF, the IRMA may yield erroneously high levels of free IGF-I.

Patients with CRF are usually characterized by in-

creased serum levels of GH [2, 3], and accordingly, we observed an approximate fourfold increase in overnight fasting serum levels of GH in patients with CRF. Veldhuis et al compared by deconvolution analysis the spontaneous 24-hour secretion of GH in adult men with hemodialysis dependent ESRF and healthy controls and observed an increased daily secretion rate as well as prolonged half-life in ESRF [5]. These observations are in agreement with findings in patients with CRF following intravenous administration of rhGH, which additionally showed that the MCR of rhGH was reduced by approximately 50% when compared with controls [4]. Furthermore, serum GHBP, which has been used as an estimate of cellular GH receptor density, is reduced in CRF [2, 6]. Thus, it has been hypothesized that the CRF-related hypersomatropinemia is caused by a reduced renal GH clearance and a relative insensitivity to the actions of GH. However, an attenuated negative feedback regulation of IGF-I on the pituitary may also be involved [5], and in this context, we find it noteworthy that levels of free but not total IGF-I were markedly decreased in patients with CRF. A recent study in healthy adults indicated that free rather than bound IGF-I was involved in the feedback inhibition of GH secretion [27]. Assuming this is also true in patients with CRF, our findings of reduced levels of free IGF-I provide a simple explanation for the increased secretion of GH in CRF.

In patients with CRF, more than 75% of the variation in serum-free IGF-I could be predicted statistically by a linear combination of levels of total IGF-I, IGFBP-1, and IGFBP-2, whereas intact as well as proteolyzed IGFBP-3 failed to show any significant relationship with free IGF-I. Furthermore, C_{Cr} correlated positively with serum free IGF-I and inversely with IGFBP-1 and -2. Thus, these data strongly support the hypothesis that CRF-related growth failure and tissue catabolism are partly caused by an increased concentration of circulating IGFBP-1 and -2, resulting in low serum levels of free IGF-I and thus IGF-I bioactivity. Furthermore, we find these results interesting in view of clinical observations in children. Thus, more than 20 years ago, Schwalbe et al showed that in serum from children with CRF, the IGF bioactivity *in vitro* correlated positively with growth velocity and glomerular filtration rate (GFR) [9]. Recently, Tönshoff et al found an inverse relationship between height SD and serum levels of IGFBP-1 and IGFBP-2, respectively, in prepubertal children with CRF, whereas levels of IGFBP-3 did not correlate with height [10]. Powell et al studied changes in IGFBPs in children with CRF before and after 12 months of treatment with rhGH, and based on the observed increment in height, they suggested that IGFBP-3, in contrast to IGFBP-2, was not likely to be a growth inhibitor [28]. The strong coincidence between these studies and ours may be considered as an indirect support for the idea that the CRF-related growth inhibition is at least partly related to an imbalance between free IGF-I and IGFBP-1 and -2.

Although our data suggest IGFBP-1 and -2 to play a central role in the regulation of free IGF-I in renal disease, we cannot preclude that a reduced production rate of IGF-I may also participate. In support of an impaired protein synthesis in CRF, we observed a 20% reduction in serum albumin levels. However, studies of the impact of renal disease on the synthesis of IGF-I have been ambiguous. Thus, based on a mathematical model, Blum et al estimated the IGF-I production rate to be reduced by an order of magnitude in children with CRF [7]. This idea has been supported by experimental studies in uremic rats showing a 60% reduction in the hepatic IGF-I mRNA expression [29], whereas pharmacokinetic studies in humans have failed to show any difference in the calculated IGF-I production rate [23]. Finally, changes in the renal excretion rate of free IGF-I may be of importance. Studies in patients with nephrotic syndrome have shown an increased urinary excretion rate of IGFBP-1 and -2 [30], and the same may be true for free IGF-I. However, in this study, neither serum albumin nor the 24-hour urinary protein excretion rate was significantly correlated with serum levels of free IGF-I (P values larger than 0.25; data not shown).

The kidneys are believed to play an important role in the removal of circulating peptides and an impaired kidney function may therefore explain the elevated circulating IGFBP levels in CRF. Supportive of this, we observed strong inverse correlations between C_{Cr} and serum levels of IGFBP-1 and -2, and similar observations were made in children with CRF [10]. On the other hand, experiments in uremic rats showed markedly elevated IGFBP-1 and -2 serum peptide and liver mRNA levels, suggesting that an increased IGFBP production was also involved [29]. In this context, we observed an inverse relationship between IGFBP-1 and serum insulin, indicating that the physiological association between these two peptides is maintained in a hyperinsulinemic and insulin-resistant condition such as CRF [3, 13, 31]. Thus, the increased levels of IGFBP-1 and -2 may not merely be secondary to changes in the renal handling of peptides.

In our study, patients with CRF showed an increased abundance of circulating low molecular IGFBP-3 fragments, whereas levels of intact IGFBP-3 did not differ from controls. This observation is in accordance with previous studies in CRF [2, 7, 32, 33]. Previous studies have failed to detect any significant IGFBP-3 proteolysis *in vitro* in serum from patients with CRF, and therefore, the accumulation of IGFBP-3 fragments has been suggested to result from reduced renal clearance [2, 32, 33]. However, in this study, the *in vitro* degradation of ^{125}I-rhIGFBP-3 was significantly increased and inversely correlated with C_{Cr} in patients with CRF. This discrepancy may be explained by differences in the selection of pa-

tients as well as in the number of subjects included. Because the *in vitro* IGFBP-3 degradation assay is in its nature semiquantitative, care should be taken when looking at differences of 7%. However, the increase was significant, and the relatively large number of subjects included in the study makes us suggest IGFBP-3 proteolysis to be operative in adults with CRF.

The biological significance of IGFBP-3 proteolysis still remains to be clarified. Studies in pregnancy have shown that the IGFBP-3 protease generates two types of fragments: a 22 to 25 kDa fragment with low ligand affinity and a smaller 16 kDa fragment, which has completely lost the ability to bind IGFs [14]. Because the ligand affinity of fragmented IGFBP-3 is markedly reduced when compared with intact IGFBP-3, IGFBP-3 proteolysis has been speculated to represent a compensatory mechanism serving to increase free IGF-I and thus IGF-I action [34–36]. On the other hand, the fragment without ligand binding has been observed to inhibit the actions of IGF-I *in vitro* [14], and IGFBP-3 proteolysis may therefore inhibit as well as stimulate IGF-action. During pregnancy and in stress situations such as following major surgery, the majority of circulating IGFBP-3 rapidly undergoes proteolytic cleavage [35, 37], and in both conditions, this has been associated with a relative increase in free IGF-I levels [35, 36]. In CRF, the enzymatic degradation of IGFBP-3 is less pronounced, and this may explain that we did not observe any significant correlation between IGFBP-3 proteolysis and serum levels of free IGF-I. Thus, the role of IGFBP-3 proteolysis as a regulator of free IGF-I appears to be less important in CRF than in pregnancy and postoperatively.

Levels of serum total IGF-I remained unchanged in patients with CRF, whereas serum total IGF-II was elevated by 40%. This observation is in accordance with previous studies [28, 38]. However, it was surprising that only free IGF-I was suppressed in CRF and that free IGF-II remained within the normal range. This observation indicates that the production rate of IGF-II is not inhibited by CRF, as it has been suggested for IGF-I [7]. The pathophysiological mechanisms responsible for the observed differences in IGF-I and IGF-II are not clear. However, nutritional factors are known to affect IGF-I and IGF-II differentially [39, 40], and therefore, the metabolic aberrations in CRF [1] may be of importance.

In conclusion, we show for the first time that serum-free IGF-I is markedly reduced in adults with CRF and that levels correlate positively with C_{Cr} and inversely with IGFBP-1 and -2. Furthermore, we observed a modest, but significantly increased IGFBP-3 proteolysis *in vivo* and *in vitro.* However, this appeared to have little impact on levels of free IGF-I. In contrast to free IGF-I, free IGF-II remained within the normal range.

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